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# Overexpression of the Chemokine Receptor CCR7 in Patients with Hepatocellular Carcinoma: Correlation with Disseminated Circulating Tumor Cells

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## ABSTRACT

Disseminated circulating tumor cells (CTCs) are cancer cells that have detached from the primary tumor and survived in the circulation, thus enable the spread of cancer from its site of origin. The chemokine receptor 7 (CCR7) has been linked to tumor dissemination and poor prognosis in solid tumors. However, its relationships with CTCs in liver cancer still not clear. This study aimed to identify the relationship between CCR7 and CTCs in hepatocellular carcinoma (HCC) patients, and to assess their predictive values as noninvasive markers. Seventy-one HCC patients and 20 normal individuals were included. CTCs were detected in the peripheral blood by flow cytometry defined as $CD45^{-}CK19^{+}CD90^{+}$cells. Expression of CCR7 was assessed by real time PCR. Clinical and routine laboratory investigations included tumor size and number of tumors detected by ultrasound. Also, alpha fetoprotein )AFP), CBC, PT, INR, ALT, AST, bilirubin, albumin, and creatinine were analyzed. Results indicated that HCC patients were classified according to their Childs-Pugh score system (CPSS) into 2 subgroups A5 (N=51) and A6 (n=20). It was found that $CCR7^{mRNA}$ increased significantly in HCC patients and its elevation was correlated with CTCs count. Besides, there were significant differences in $CCR7^{mRNA}$ and in CTCs between the studied groups. Both CCR7 and CTCs were significantly correlated with age, levels of ALT, and AST and negatively with platelets and serum albumin. $CCR7^{mRNA}$ was correlated significantly with total bilirubin and tumor size, while CTCs was significantly correlated with AFP and INR. No significant difference between both groups regarding kidney function tests. The diagnostic efficiency of CTCs and CCR7 was assessed using ROC curve, where it was clear that CCR7 at cut off >1.02 could discriminate between patients and control with 93.1% sensitivity, 78.8% specificity, 88.5% PPV and 86.7% NPV, while CTCs concentration> 3.5 is the cutoff between patient and control groups with 91.4% sensitivity, 81.8% specificity, 89.8% PPV and 84.4 % NPV. The current results indicated that each of CCR7 and CTCs can be used as an efficient diagnostic marker, and both complement in reflecting different liver reserve status. Conclusion: CCR7 expression is increased with HCC progression. The combined assessment of CTCs and CCR7 could be considered as potential noninvasive biomarkers for HCC progression. Additional research with a greater number of patients is needed on this topic.

***Keywords*:** Hepatocellular carcinoma, circulating tumor cells, Chemokine receptor seven, polymerase chain reactions, flow cytometry.

## INTRODUCTION

HCC is the sixth most common neoplasm and represents a major health challenge with an annual global death > 600,000 which makes it the third most lethal cause worldwide (Huh *et al*., 2002). In Egypt, studies revealed that about ninety

percent of HCC cases are HCV-related (El-Kassas and Elbadry, 2022).

The lethality of HCC is linked increasingly with early metastasis, which is emerging from dissemination of tumor cells (DTCs) from the original tumor or metastatic foci that are flowing freely in the blood circulation. These calls are considered the drivers of recurrence and metastasis following liver cancer surgery for primary HCC (Ahn *et al*., 2021). These cells are often detectable in the peripheral blood as circulating tumor cells (CTCs). CTCs can lead to a new fatal metastasis and can be vividly described as "seeds" of tumors. CTCs-positive rate was directly correlated with tumor size and counts as a biomarker of poor prognosis. The absolute numbers of CTCs detected have been associated with survival and treatment response and associated with increased recurrence risk after resection and shorter overall survival as the more advanced the cancer stage, the higher number of these cells in the peripheral blood (Ou *et al*., 2018).

One of inflammation-related cancer is hepatocellular carcinoma, these chronic infections with hepatitis viruses (HBV and HCV) and the sustained inflammatory reactions related to the infections represent major risk factors for HCC development (Han *et al*., 2015). However, the chronic inflammation is characterized by the continued expression of chemokines that enables the recruitment of immune cells to the liver. When inflammatory cells are activated, they release free radicals, such as reactive oxygen species (ROS) and nitric oxide (NO) reactive species, which may cause DNA damage and cause gene mutations, thus promoting neoplastic transformation (Ma *et al*., 2015).

Chemokines play a vital role in tumor progression, in several metastatic tumors including HCC and colorectal liver metastasis (Jiao *et al*., 2019). Currently, chemokines and their receptors such as the-CCR7 axis have received much research interest because it has been linked to poor prognosis and tumor dissemination because of induction of the process of epithelial-mesenchymal transition in gastric as well as ovarian cancer (Cheng *et al*., 2014). In addition, the CCL21-CCR7 axis was reported as an important regulator of growth and progression of esophageal cancer, as there is a strong association between the levels of their expression and the degrees of differentiation (Goto *et al*., 2019). Although many studies indicated that CCR7 are associated with dissemination of HCC cells (Schimanski *et al*., 2006), however, its relationships with CTCs and progression of HCC remains not clear. Therefore, in the present study, we aimed to assess the contribution of CTCs and CCR7 as noninvasive biomarkers for patients with HCC with distinct stages of the diseases.

## PATIENTS AND METHODS

### Study design and population:

The study sample includes 71 Egyptian HCC patients diagnosed by liver biopsy, CT scan, or MRI and twenty (age and gender matched) healthy subjects (control group). All subjects were recruited from the Outpatient Clinics and Inpatient of internal medicine Department of Ain-Shams University Hospitals. The laboratory investigations enumeration of CTCs and mRNA expression CCR7 were performed at the Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt. All patients were subjected to through medical history and clinical examination. Laboratory investigations were performed for all participants.

### **Sample collection** and **preparation:**

Blood samples were collected by venipuncture in heparin BD Vacutainer tubes and were divided into two halves, one for routine lab work and the other part for separation of the Peripheral Blood Mononuclear cells "PBMCs" that were used in RT-PCR and Flow Cytometry analyses. Buffy coats from peripheral blood samples

were separated carefully using Ficoll-Paque plus density (1077 g/L) using "Amersham biosciences Kit" according to the manufacturer's instructions.

**Routine laboratory investigations**

Complete blood count (CBC) was performed by 5-part differential automated cell counter Beckman Coulter® LH 750 (Coulter Corporation, Florida, USA), prothrombin time (PT, INR) was done on fully automated blood coagulation analyzer STA Compact Max-Stago (Asnieres Sur Seine Cedex, France). Serum ALT, AST, total bilirubin, direct bilirubin, albumin, Alpha fetoprotein (AFP), and serum creatinine were confirmed on Beckman coulter AU 480 system (Beckman coulter, Inc. 250s. Kraemer Blvd. Brea, CA92821, USA).

**Estimation of chemokine receptor 7 (CCR7) by real time PCR:**

RNA was extracted using the "Pure link RNA mini kit "supplied by "Ambion" (Life technologies, Carlsbad, USA) following the manufacturer's procedure. The extracted RNA samples were eluted aliquoted into sterile tubes and stored at -80ºC until further processing. The extracted RNA was then converted to cDNA using the "High-capacity cDNA reverse transcription kit" from "Applied Biosystems" (Life technologies, Carlsbad, USA) according to the manufacturer's instructions. Quantitative real-time PCR was done using a Light Cycler System (DT prime thermal cycler) (DNA Technology, Moscow, Russia) with CCR7-specific primers (forward): 5′-CATGCTCCTACTTCTTTGCATC-3′, and (reverse) 5′-CACTGTGGCTAGTATCCAGATG-3′ Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward): 5'-ACCCAGAAGACTGTGGATGG-3'. (Reverse) : 5'-TCTAGACGGCAGGTCAGGT-3' was amplified as an internal control "Ambion" (Life technologies, Carlsbad, USA). The PCR mix contained 3.2 µL Nuclease free H2O, 0.8 dNTP mix, 2 µL RT random primer, 1µl multiscribe$^{TM}$ reverse transcriptase, 2 µl RT buffer and 1µL RNase inhibitor. Samples with the master mix were placed into the rotor of the Light Cycler for amplification. PCR cycles were performed using the "Maxima SYBR Green q PCR master mix" supplied by "Thermo Fisher Scientific" (Life technologies, Carlsbad, USA). CCR7 mRNA concentration was calculated using the delta-delta Ct method, also known as the 2–ΔΔCt method, in which the following equation was used: CCR7 RNA concentration = 2Δct (Δ ct = CCR7 reading – GAPDH reading) to calculate the relative fold CCR7 gene expression of samples (Livak and Schmittgen, 2001).

**Identification of CTCs by Flow Cytometry**

Flow cytometric measurement of CTCs was performed using "BD Accuri C6 plus" flow cytometry (BD Life sciences Inc, USA). Anti-human anti-CD45, anti-human CD90 and anti-CK19 monoclonal antibodies were used to identify CTCs as cells negative for CD45 and positive for CK19 and CD90 ($CD45^{-}CK19^{+}CD90^{+}$) in the separated mononuclear layer. The fluorescence of the circulating tumor cells is analyzed to differentiate between the positively stained cells from the negative unstained ones using direct flow cytometry staining method as previously reported (Elshal *et al*., 2016). The results were then expressed as numbers of $CD45^{-}$CK19+CD90+ CTCs in relation to all cells acquired by the cytometer.

**Statistical analysis**

Results were detected using SPSS version 24. Quantitative data were examined as mean ± standard error of means (SEM). Qualitative data were expressed as frequency and percentage. Chi-square test was used to compare qualitative variables. ANOVA test was used

to compare more than two groups with Bonferroni post hoc analysis for variance between pair groups. The relationship between variables in the same group was evaluated using Spearman's correlation co-efficient test. Receiver-operating characteristic (ROC) curve analysis was used to examine the value of CTCs and CCR7 for discrimination between cases and controls. A p value ≤ 0.05 was considered statistically significant.

## RESULTS

### Clinical characteristics:

Ninety-one subjects that enrolled in the study were categorized into 3 groups: (patient group) 71 HCC patients [6 (8.5%) males and 65(91.5%) females]. The patients' ages ranged from 50- 61 years old with mean of 56.20 ± 5.53 years. Twenty age and gender-matched healthy subjects were recruited as control group, [0 (0%) male and 20 (100%) female]. Their ages ranging from 31- 42 years with mean of 37.00 ± 5.22 years. HCC patients were grouped according to their Childs-Pugh class into 2 groups A5 (N=51) and A6 (n=20). The clinical features and laboratory findings of the studied groups are shown in Table (1). A comparative study by ANOVA test showed statistically significant difference in all studied parameters except for total bilirubin, creatinine, and total lymphocytes count.

Real time PCR of CCR7 mRNA:

CCR7 expression was found significantly higher in the HCC-A5 and HCC-A6 patients groups compared to healthy controls (P < 0.002) (Table 1).

**Table 1: Clinical characteristics and laboratory findings of the studied groups.**

| Parameter | Healthy | | Child-Pugh A5 | | Child-Pugh A6 | | ANOVA | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | F-value | P-value |
| **Age** | 47.5 | 5.25 | 55.47 | 4.91 | 60.44 | 6.52 | 9.34 | 0.074 |
| **ALT U/L** | 28.05 | 1.773 | 39.24 | 3.015 | 45.54[a] | 6.148 | 3.316 | 0.041 |
| **AST U/L** | 27.10 | 1.676 | 44.72[a] | 3.306 | 51.08[a] | 5.380 | 6.261 | 0.003 |
| **T. Bilirubin (mg/dL)** | 1.05 | 0.050 | 1.16 | 0.059 | 1.38 | 0.140 | 2.525 | 0.086 |
| **D. Bilirubin (mg/dL)** | 0.25 | 0.099 | 0.36[a] | 0.064 | 0.69[a] | 0.175 | 3.258 | 0.043 |
| **Albumin (g/dL)** | 4.15 | 0.082 | 3.50[a] | 0.086 | 3.38[a] | 0.241 | 8.732 | 0.0001 |
| **INR** | 1.00 | 0.000 | 1.12 | 0.043 | 1.31[a] | 0.133 | 3.678 | 0.029 |
| **Creatinine (mg/dL)** | 1.00 | 0.000 | 1.00 | 0.000 | 1.08[a] | 0.077 | 3.143 | 0.068 |
| **Hb (Hb) (g/dl)** | 13.10 | 0.161 | 12.45 | 0.181 | 11.69 | 0.499 | 4.434 | 0.015 |
| **T. lymph. (x $10^3$/µl)** | 6.00 | 0.423 | 5.57 | 0.219 | 5.15 | 0.807 | 0.784 | 0.46 |
| **Platelets (x $10^3$/µl)** | 273.95 | 10.910 | 191.02[a] | 10.27 | 137.46[a,b] | 24.37 | 14.98 | 0.0001 |
| **AFP (ng/ml)** | 1.75 | 0.33 | 57.25[a] | 8.53 | 93.25[a,b] | 13.27 | 10.49 | 0.0001 |
| **Tumor size (cm)** | - | - | 2.76 | 0.176 | 3.85[b] | 0.553 | 5.766 | 0.019 |
| **CCR7 (folds)** | 0.25 | 0.099 | 32.33[a] | 5.572 | 36.50 [a] | 4.95 | 6.859 | 0.002 |
| **CTCs (count/ml)** | - | - | 8.79[a] | 0.713 | 12.62[a,b] | 1.328 | 27.34 | 0.0001 |

Values expressed as mean ± standard error of means (SEM) (n=20) for healthy control and (n=51 and 20) for A5 and A6 class HCC patients. Level of significance at p<0.05.A comparative study by ANOVA with Bonferroni post hoc test. a: significant (P<0.05) compared with healthy controls, b: significant (P<0.05) compared with HCC patients with child Pugh class A5.

### CTCs enumeration by flow cytometry:

CTCs enumeration was done using flow cytometry as $CD45^{-}CK19+$ cells (Fig. 1), and it was found significantly higher in A6 HCC patients compared to A5 HCC and the controls (P ≤ 0.0001).

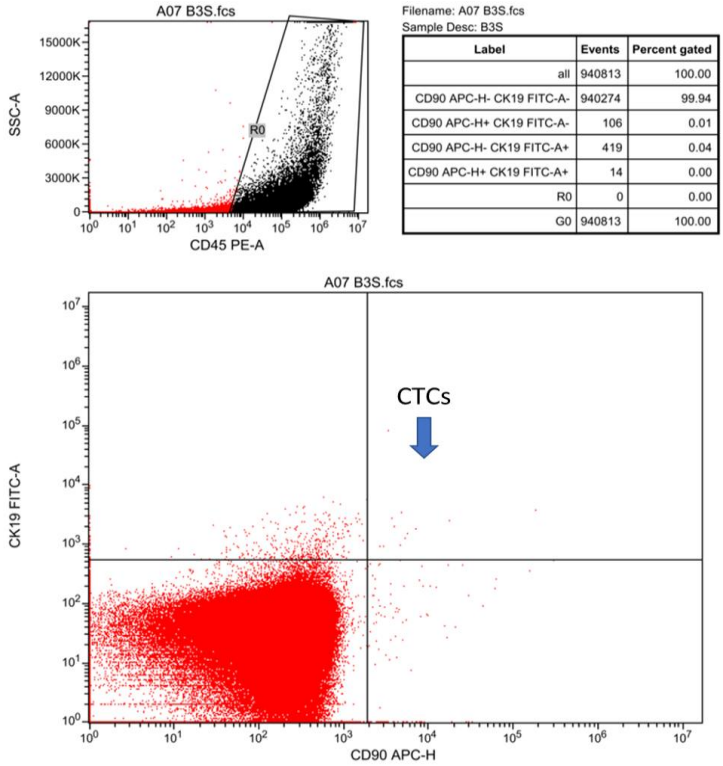



**Fig. 1. Graphical representation of the Flow cytometry analysis of CTCs ($CD45^{-}CK19^{+}CD90^{+}$) cells circulating in the blood. The arrow points to the circulating tumor cells population as detected as CK19+CD90+ cells after exclusion of $CD45^{+}$ blood cells.**

**Correlations between biochemical parameters:**

There was a significant positive correlation between mRNA expression of CCR7 and the numbers of CTCs ($p<0.05$) (Fig. 2). CCR 7 expression levels also positively correlated with tumor size and liver functions tests ALT, AST, and T. bilirubin. Moreover, CCR7 mRNA expression was found negatively correlated with serum albumin and blood platelets (Table 2). On the other hand, there were significant positive correlations between CTCs and age, ALT, INR, and AFP. While there were significant negative correlations between CTCs and Alb and Platelet, with r values of -0.311 and -0.334 respectively ($p< 0.05$) (Table 2).

**Fig. (3): Correlation between the CTCs and CCR7 expression levels.**

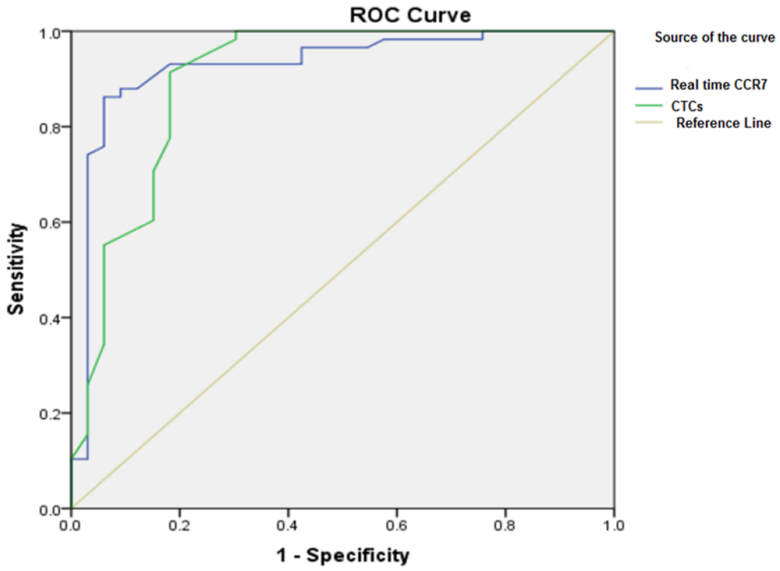
**Table 2: Correlation between the CCR7 and different variables in case group**

| Parameter | CCR7 | | CTCs | |
|---|---|---|---|---|
| | r | Sig. | r | Sig. |
| **Age** | 0.277* | 0.012 | 0.616** | 0 |
| **ALT U/L** | 0.331** | 0.002 | 0.269* | 0.01 |
| **AST U/L** | 0.280** | 0.01 | 0.219* | 0.043 |
| **Total Bili. (mg/dL)** | 0.244* | 0.025 | 0.114 | 0.283 |
| **Direct Bilirubin (mg/dL)** | 0.18 | 0.101 | 0.077 | 0.473 |
| **Albumin (g/dL)** | -.310** | 0.004 | -0.311** | 0.003 |
| **INR** | 0.163 | 0.138 | 0.209* | 0.048 |
| **Creatinine (mg/dL)** | -0.072 | 0.514 | 0.094 | 0.379 |
| **Hb (g/dl)** | -0.17 | 0.123 | -0.054 | 0.614 |
| **T. Lymph (x $10^3$/µl)** | -0.19 | 0.084 | -0.06 | 0.575 |
| **Platelets (x $10^3$/µl)** | -0.249* | 0.022 | -0.334** | 0.001 |
| **AFP ng/ml** | 0.09 | 0.422 | 0.309** | 0.004 |
| **Tumor size (cm)** | 0.412** | 0.001 | 0.053 | 0.662 |
| **CTCs count/ml** | 0.258* | 0.019 | 1 | |

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

## Diagnostic performance of CCR7 and CTC

Using ROC curve, it was shown that CCR7 at the cut-off > 1.02 can be used to discriminate between patients and controls with 93.1% sensitivity, 78.8% specificity, 88.5% PPV and 86.7% NPV, while CTC concentration > 3.5 was the cut-off between patients and controls with 91.4% sensitivity, 81.8% specificity, 89.8% PPV and 84.4 % NPV (Fig. 3).




| Parameter | Cut off | AUC | Sensitivity | specificity | PPV | NPV | P-value |
|---|---|---|---|---|---|---|---|
| CCR7 | >1.020 | 0.927 | 93.1 | 78.8 | 88.5 | 86.7 | 0.01 |
| CTCs | >5.30 | 0.900 | 91.4 | 81.8 | 89.8 | 84.4 | 0.01 |

**Fig. 3: Receiver operating characteristic (ROC) curve between patient and control group as regards CCR7 and CTCs.**

## DISCUSSION

Chemokines, as small chemotactic cytokines, participates in much physiological function on the cell expressing their receptor; they play a vital role in the progression of HCC, as CCR7 was demonstrated mediates epithelial-mesenchymal transition and suppressing apoptosis through AKT pathway (Xu *et al*., 2017). Additionally, the CCL21-CCR7 axis have received much attention in HCC research stemmed from the previous work by Schimanski *et al*., (Schimanski *et al*., 2006) who reported that these circulating tumor cells are disseminated from the primary tumor in CCR7-positive HCC patients. Furthermore, Chen et al., (Shen *et al*., 2022) demonstrated that CCL21-CCR7 axis is a potential therapeutic target for blocking the progression of HCC.

Several HCC staging systems have been proposed for the assessment of patient's prognosis and response to treatment (Liu *et al*., 2016), however survival in HCC patients was found not only dependent on stages of tumor, but also the liver functional reserve (Kinoshita *et al*., 2012). One of the scores that rely mainly on estimating the liver functional reserve is Child–Pugh scoring system (CPSS) (Wang *et al*.,2018). Interestingly, it was reported that the survival rates are different in each CPSS grade and associated with treatment response for early stages of HCC (Roberts *et al*., 2018). Indeed, CPSS system that has proved to be adequate for the stratification of staging systems given that the degree of liver dysfunction is one of the most important prognostic factors for HCC (Kemp *et al*., 2005). Additionally, Hung et. Al., (Hung *et al*., 2014) demonstrated that HCC group of patients with A5 score had a better overall survival rate than those with A6 group as regard the early tumor stage and higher rate of effective curative treatments.

Therefore, in the present study we investigate whether the expression of CCR7 is associated with disseminated tumor cells measured as CTCs and with other prognostic markers in HCC with Grades A5 and A6 CPSS. In our study, fifty-one patients were CPSS class A5, whereas 21 patients were class A6. After adjustment for the clinical influence factors of gender and age, we found significantly higher expression of CCR7 associated with the significant increase CTCs compared with healthy controls and through the progression of liver cancer from A5 to A6.Although there were no significant differences in CCR7 expression of CPSS class A5/A6, however CTCs and serum AFP showed significant differences between A5/A6 grades. This result goes in accordance with the studies conducted by Qin *et al*. (Qin *et al*., 2021) who established a study included many patients suffering from HCC in which elevated levels of CCR7 was found as a prognostic factor to distinguish them from those with no detected focal lesion.

The finding that of CCR7 expression is upregulated as early as CPSS grade A, on contrary to CTCs and AFP, suggests that it can be an early event for liver tumorigenesis. This suggestion is supported by the research of Zhou *et al*., (Zhou *et al*., 2022) who noticed that serum levels of CCR7 is considered a less reliable marker of HCC as its elevated levels may be related to other GIT tumors as colon cancer. Moreover, it was emphasized that CCR7 is not a specific biomarker for liver tumors as its elevations were also reported in other cancers as colorectal cancers and colorectal liver metastases (Wu *et al*., 2022).

Disseminated CTCs, the cells that derive from the primary or metastatic lesions and migrate into circulation and, are regarded as the "seeds" of tumor metastasis that are increased in patients with HCC with advanced stages (Schimanski *et al*., 2006). We observed a statistically significant difference regarding CTCs detected by flow cytometry between patients with HCC

group A5 as compared to group A6 ($p<0.05$), and also regarding detection of focal lesion by ultrasound. This agrees with Qi *et al*., (Qi *et al*., 2018) who reported that elevated level of CTCs and thus positive focal lesions in liver are detected in most of HCC patient. Our data also revealed the presence of positive correlation of CTCs with AST, ALT, AFP, and INR, this comes in accordance with Qi *et al.,* (Qi *et al*., 2018) who additionally added that the increase in serum CTCs concentrations as in HCC is associated with affection of liver functions so it could be used for differentiation between patients with HCC and healthy individuals. Meanwhile, our results confirmed the presence of negative correlation between albumin level and platelet count and CTCs where albumin levels were low and platelet count which agrees with Haruki *et al*., (Haruki *et al*., 2018) who proved that serum level of albumin as well platelet count is decreased in patients suffering of HCC with elevated level of CTCs. This supports the previously published concept that the enumeration of CTCs with exclusive and specific biomarkers may provide better diagnostic and individualized treatment options for patients with HCC (Chen *et al*., 2020).

CCR7 mRNA levels were also significantly increased in HCC patients, and they were correlated with CTCs numbers and tumor size and grade. To examine the diagnostic efficiency of CTCs and CCR7, ROC curve was done between CCR7 and CTCs in discriminate between patient and control groups which revealed that CCR7 at cut off >1.02 could discriminate between patients and control with 93.1% sensitivity, 78.8% specificity, 88.5% PPV and 86.7% NPV, while CTCs concentration> 3.5 is the cutoff between patient and control groups with 91.4% sensitivity, 81.8% specificity, 89.8% PPV and 84.4 % NPV. These data indicated that each of CCR7 and CTCs is efficient diagnostic marker, and both complement in reflecting different liver reserve status. However, the pronounced role of CTCs with the expression of CCR7 in liver cancer has not been documented in the early stage (CPSS A5/A6) of HCC to the best of our knowledge.

## Conclusion

CCR7 expression is increased with HCC progression. The combined assessment of CTCs and CCR7 could be considered as potential noninvasive biomarkers for HCC progression. Additional research with a greater number of patients is needed on this topic.

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## REFERENCES

Ahn, J.C.; Teng, P.C.; Chen, P.J.; Posadas, E., et al., ( 2021). Detection of circulating tumor cells and their implications as a biomarker for diagnosis, prognostication, and therapeutic monitoring in hepatocellular carcinoma. Hepatology, 73(1):422-436.

Chen, F.; Zhong, Z.; Tan, H.-Y.; Wang, N. and Feng, Y. (2020). The significance of circulating tumor cells in patients with hepatocellular carcinoma: Real-Time Monitoring and Moving Targets for Cancer Therapy. Cancers 12(7):1734.

Cheng, S.; Han, L.; Guo, J.; Yang, Q.; Zhou, J. and Yang, X. (2014). The essential roles of CCR7 in epithelial-to-mesenchymal transition induced by hypoxia in epithelial ovarian carcinomas. Tumor Biology, 35(12):12293-12298.

El-Kassas, M. and Elbadry, M. (2022). Hepatocellular Carcinoma in Africa: Challenges and Opportunities', Frontiers in Medicine 9.

Elshal, M.F.; Aldahlawi, A.M.; Saadah, O.I. and McCoy, J.P. (2016). Expression of CD200R1 and its Ligand CD200 on T-helper lymphocytes of pediatric patients with ulcerative colitis and crohn's disease. Clinical Laboratory, 62(8):1521.

Goto, M. and Liu, M. (2019). Chemokines and their receptors as biomarkers in esophageal cancer. Esophagus, 17(2):113--121.

Han, K.Q.; He, X.Q.; Ma, M.Y.; Guo, X.D.; Zhang, X.M.; Chen, J.; et. al. (2015). Inflammatory micro-environment and expression of chemokines in hepatocellular carcinoma. World J. Gastroenterol., WJG, 21(16):4864.

Haruki, K.; Shiba, H.; Saito, N.; Horiuchi, T.; Shirai, Y.; Fujiwara, Y.; Furukawa, K.; Sakamoto, T. and Yanaga, K. (2018). Risk stratification using a novel liver functional reserve score of combination prothrombin time international normalized ratio to albumin ratio and albumin in patients with hepatocellular carcinoma. Surgery 164(3):404-410.

Huh, Y.; Cho, Y.J. and Nam, G.E. (2022). Recent Epidemiology and Risk Factors of Nonalcoholic Fatty Liver Disease. J. Obes. Metab. Syndr., 31(1):17-27.

Hung, H.-H.; Chao, Y.; Chiou, Y.-Y.; Li, C.-P.; Lee, R.-C.; Huo, T.-I.; Huang, Y.-H.; Chau, G.-Y.; Su, C.-W. and Yeh, Y.-C. (2014). A comparison of clinical manifestations and prognoses between patients with hepatocellular carcinoma and Child–Pugh scores of 5 or 6. Medicine, 93(29).

Jiao, X.; Shu, G.; Liu, H.; Zhang, Q.; Ma, Z.; Ren, C.; Guo, H.; Shi, J.; Liu, J.; Zhang, C. and Wang, Y., (2019). The diagnostic value of chemokine/chemokine receptor pairs in hepatocellular carcinoma and colorectal liver metastasis. J. Histochem. Cytochem., 67(5):299-308.

Kemp, W.; Pianko, S.; Nguyen, S.; Bailey, M.J. and Roberts, S.K. (2005). Survival in hepatocellular carcinoma: Impact of screening and etiology of liver disease. J. Gastroenterol. Hepatol., (Australia), 20(6): 873–881.

Kinoshita, A.; Onoda, H.; Imai, N.; Iwaku, A.; Oishi, M.; Fushiya, N. et al., (2012). Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. British J. Cancer, 107(6):988-993.

Liu, P.H.; Hsu, C.Y.; Hsia, C.Y.; Lee, Y.H.; Su, C.W.; Huang, Y.H., et al., (2016). Prognosis of hepatocellular carcinoma: assessment of eleven staging systems. J. Hepatol., 64(3):601-608.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25(4):402-408.

Ma, H.; Gao, L.; Li, S.; Qin, J.; Chen, L.; Liu, X. et al. (2015). CCR7 enhances TGF-β1-induced epithelial-mesenchymal transition and is associated with lymph node metastasis and poor overall survival in gastric cancer. Oncotarget, 6(27): 24348.

Ou, H.; Huang, Y.; Xiang, L.; Chen, Z.; Fang, Y.; Lin, Y.; Cui, Z.; Yu, S.; Li, X. and Yang, D. (2018). Circulating tumor cell phenotype indicates poor survival and recurrence after surgery for hepatocellular carcinoma. Digestive Diseases Sci., 63(9):2373-2380.

Qi, L.N.; Xiang, B.D.; Wu, F.X.; Ye, J.Z.; Zhong, J.H.; Wang, Y.Y.; et al. (2018). circulating tumor cells

undergoing EMT provide a metric for diagnosis and prognosis of patients with hepatocellular carcinoma CTCs in patients with HCC. Cancer Res., 78(16): 4731-4744.

Qin, L.; Kuai, J.; Yang, F.; Yang, L.; Sun, P.; Zhang, L. and Li, G. (2021). A novel CCR7-related immune prognostic signature for liver hepatocellular carcinoma (LIHC) based on immunogenomic profiling. Research Square; 2021. Preprint. DOI: 10.21203/rs.3.rs-154215/v1.

Roberts, S. K.; Gazzola, A.; Lubel, J.; Gow, P.; Bell, S.; Nicoll, A.; et al. (2018). Treatment choice for early-stage hepatocellular carcinoma in real-world practice: impact of treatment stage migration to transarterial chemoembolization and treatment response on survival. Scandinavian J. Gastroenterol. 53(10-11):1368-1375.

Schimanski, C.C.; Bahre, R.; Gockel, I.; Junginger, T., et al. (2006). Chemokine receptor CCR7 enhances intrahepatic and lymphatic dissemination of human hepatocellular cancer. Oncology reports, 16(1):109-113.

Shen, C., Li, J., Li, R., Ma, Z., Tao, Y., Zhang, Q., & Wang, Z. (2022). Effects of Tumor-Derived DNA on CXCL12-CXCR4 and CCL21-CCR7 Axes of Hepatocellular Carcinoma Cells and the Regulation of Sinomenine Hydrochloride. Frontiers in Oncology, 12.

Wang, Y.Y.; Zhao, X.H.; Ma, L.; Ye, J.Z.; Wu, F.X., Tang, J.; et al., (2018). Comparison of the ability of Child-Pugh score, MELD score, and ICG-R15 to assess preoperative hepatic functional reserve in patients with hepatocellular carcinoma. J. S.urg. Oncol., 118(3):440-445.

Wu, Y.; Yang, S.; Ma, J.; Chen, Z.; Song, G.; Rao, D.; Cheng, Y.; et al., (2022). spatiotemporal immune landscape of colorectal cancer liver metastasis at single-cell level. Cancer Discovery 12(1):134-153.

Xu, B.; Zhou,M.; Qiu, W.; Ye, J.; Feng, Q. (2017). CCR7 mediates human breast cancer cell invasion, migration by inducing epithelial-mesenchymal transition and suppressing apoptosis through AKT pathway. Cancer Med, 6 (5): 1062-1071

Zhou, H.; Liu, Z.; Wang, Y.; Wen, X.; Amador, E.H.; Yuan, L.; Ran, X.; Xiong, L.; Ran, Y.; Chen, W. and Wen, Y. (2022). Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduction and Targeted Therapy, 7(1).

# Overexpression of the Chemokine Receptor CCR7 in Patients with Hepatocellular Carcinoma: Correlation with Disseminated Circulating Tumor Cells

## الإفراط في التعبير عن مستقبلات Chemokine CCR7 في المرضى الذين يعانون من سرطان الخلايا الكبدية: الارتباط مع خلايا الورم المنتشرة

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**المستخلص**

الخلايا السرطانية المنتشرة (CTCs) هي الخلايا السرطانية التي انفصلت عن الورم الرئيسي وبقيت على قيد الحياة في الدورة الدموية، وبالتالي تمكن من انتشار السرطان من موقعه الأصلي. تم ربط المستقبل الكيميائي 7 (CCR7) بانتشار الورم وضعف التشخيص في الأورام الصلبة. ومع ذلك، فإن علاقاته مع CTCs في سرطان الكبد لا تزال غير واضحة. هدفت هذه الدراسة إلى تحديد العلاقة بين CCR7 و CTCs في مرضى سرطان الخلايا الكبدية، وتقييم قيمهم التنبؤية كواسمات غير باضعة. تم تضمين واحد وسبعين مريضا من سرطان الكبد و 20 شخصا عاديا. تم اكتشاف CTC في الدم المحيطي عن طريق قياس التدفق الخلوي المحدد على أنه خلايا $CD45^{-}CK19^{+}CD90^{+}$. تم تقييم التعبير عن CCR7بواسطة PCR في الوقت الحقيقي. تضمنت الفحوصات المخبرية السريرية والروتينية حجم الورم وعدد الأورام التي تم الكشف عنها بواسطة الموجات فوق الصوتية، كما تم تحليل بروتين ألفا الجنيني (AFP) ، وCBC، و PT ، و INR، و ALT ، و AST ، والبيليروبين ، والألبومين ، والكرياتينين. النتائج: تم تصنيف مرضى سرطان الكبد وفقًا لنظام درجات Childs-Pugh (CPSS) إلى مجموعتين فرعيتين A5 (N = 51) و A6 (n = 20). كشفت النتائج أن CCR7 mRNA زاد بشكل ملحوظ في مرضى سرطان الكبد، وكان ارتفاعه مرتبطًا بعدد CTCs ، إلى جانب ذلك ، كانت هناك اختلافات كبيرة في CCR7 mRNA وفي CTCs بين المجموعات المدروسة. يرتبط كل من CCR7 و CTCارتباطًا وثيقًا بالعمر و ALT و AST وسلبًا مع الصفائح الدموية وألبومين المصل. ارتبط CCR7 mRNA بشكل كبير مع إجمالي البيليروبين وحجم الورم، بينما ارتبط CTCs بشكل كبير مع AFP و INR. لا يوجد فرق كبير بين المجموعتين فيما يتعلق باختبارات وظائف الكلى. تم تقييم الكفاءة التشخيصية لـ CTCs و CCR7 باستخدام منحنى ROC، وكشف أن CCR7 عند قطع> 1.02 يمكن أن يميز بين المرضى والتحكم بحساسية 93.1٪، وخصوصية 78.8٪ ، و 88.5٪ PPVو 86.7٪ NPV، بينما تركيز CTCs> 3.5 هو الحد الفاصل بين مجموعات المرضى ومجموعة المراقبة بحساسية 91.4٪ ، وخصوصية 81.8٪ ، و 89.8٪ PPVو 84.4٪ NPV. حيث أشارت البيانات إلى أن كل من CCR7 و CTCs هي علامة تشخيصية فعالة وكلاهما مكمل في عكس حالة احتياطي الكبد المختلفة. لذلك يمكن استنتاج أن التقييم المشترك لـ CTCs و CCR7 يمكن اعتباره مؤشرات حيوية غير باضعة محتملة لمرضى سرطان الكبد. هناك حاجة إلى مزيد من البحث مع عدد أكبر من المرضى حول هذا الموضوع.